Distribution of L-tryptophan in normal and glucose - loaded mice

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Summary. L-tryptophan is an essential amino acid in food, but is also widely used as a drug on the basis of several physiological actions. Lately, tryptophan's uses as a drug and as a food supplement have been discontinued in several countries due to its severe side-effects.

In the present study, the distribution of tryptophan in mice was studied with special attention on the target organs, where the drug has been shown to have pathological or physiological effects.

The results showed that several organs took up tryptophan and that glucose loading increased the accumulation. An interesting finding was that the highest concentration of tryptophan was found in the pancreas. The hypophysis and adrenal glands were also sites of accumulation. Within the brain the highest accumulation was found in the cerebrum. High concentrations were also seen in the gastrointestinal tract and bone marrow.

The connection between the accumulation of tryptophan and its normal and pathophysiological effects is discussed.

Keywords: Amino acid - Whole-body autoradiography - L-Tryptophan - Densitometric image analysis - Mouse

Introduction

Tryptophan is nutritionally an essential aromatic amino acid and one of the most critical amino acids in micro-organisms and the mammalian body. Having the lowest tissue concentration of all the amino acids usually renders it the rate-limiting component of protein synthesis (Munro, 1969). In addition, active neurotransmitters such as 5-hydroxytryptamine (serotonin) are formed from tryptophan in the brain. Quantitatively the most important pathway of tryptophan is its degradation via the kynurenine in the liver.

Tryptophan has been widely marketed as a non-prescription drug in the USA since 1974. Individuals have been reported to take it for sleeping
difficulties, depression or anxiety in rather large daily doses of up to 15 grams. Several recent reports refer to the serious disease called the eosinophilia-myalgia syndrome (EMS) which has appeared after using tryptophan for some weeks or up to several years. In some cases, the symptoms have begun several weeks after discontinuing the medication. EMS has been recognized since 1989 as a malady characterized by peripheral eosinophilia with scleroderma-like features (CDC, 1989, a,b; CDC, 1990; Silver et al., 1990). EMS is now reported in the United States to affect more than 1500 persons, about thirty of whom have succumbed. The latest reports, however, suggest that it was the bacterial contamination (Yamaoka et al., 1991) or chemical contaminant (Driskell et al., 1992; Ito et al., 1992) during manufacture of the product followed by autoimmune disease in the patients which caused the EMS (Criswell and Sack, 1991).

Tryptophan has been shown in many studies to cause hypoglycemia (Smith and Pogson, 1977; McDaniell et al., 1973). It also affects fatty acid synthesis (Fears and Murrell, 1980). Tryptophan is a powerful amino acid; it has been suspected of causing liver injuries in large doses (Sidransky, 1986), and even cancer (Trulson and Sampson, 1987). However, the evidence of its role in liver tumorgenesis is conflicting.

Tryptophan has been shown significantly to affect the hormones of the hypophyseal-adrenal axis (Modlinger et al., 1979, 1980; Träskman-Bendz et al., 1986).

In view of the variety of actions of tryptophan in different organs, the purpose of the present investigation was to study its tissue distribution in the body. Because glucose is known to have an effect on amino acid uptake, and proteins are usually taken with carbohydrates, we also studied tryptophan distribution when glucose was given simultaneously with tryptophan. The accumulation of tryptophan was measured in various organs using computer-assisted image analysis.

**Materials and methods**

*Animals*

The experimental animals were six male NMRI mice (30 g), maintained under constant light periods (light 14 h, dark 10 h) and with free access to food (standard diet from Hankkija Ltd. Turku, Finland) and water. L-[5-^3^H] Tryptophan (specific activity 30.0 Ci/mmol, code TRK 460) was purchased from Amersham, U.K. and carboxymethylcellulose (CMC) (Ph. Nord grade). Hexan (n-hexane), ethylether and glucose were p.a. grade from E. Merck, Darmstadt, Germany.

*Autoradiographic methods*

The whole-body autoradiographic method used has been described earlier (Sainio and Sainio, 1991). The autoradiograms were scanned on a light box with a CCD video camera (WV-CD130L/G, Panasonic, Japan). Focus and exposure were kept standardized. Images were analyzed by a MicroScale TC program with a resolution of 720 × 512 with 256 grey values. The micro-computer used in analysis was the IBM compatible EKTACO EW-286PC-02 (Estonia). Point measurements were made and standards used for each exposure.